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Mapping of Quantitative Trait Loci for Grain Iron and Zinc Concentration in Diploid A Genome Wheat

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Abstract

Micronutrients, especially iron (Fe) and zinc (Zn), are deficient in the diets of people in underdeveloped countries. Biofortification of food crops is the best approach for alleviating the micronutrient deficiencies. Identification of germplasm with high grain Fe and Zn and understanding the genetic basis of their accumulation are the prerequisites for manipulation of these micronutrients. Some wild relatives of wheat were found to have higher grain Fe and Zn concentrations compared with the cultivated bread wheat germplasm. One accession of *Triticum boeoticum* (pau5088) that had relatively higher grain Fe and Zn was crossed with *Triticum monococcum* (pau14087), and a recombinant inbred line (RIL) population generated from this cross was grown at 2 locations over 2 years. The grains of the RIL population were evaluated for Fe and Zn concentration using atomic absorption spectrophotometer. The grain Fe and Zn concentrations in the RIL population ranged from 17.8 to 69.7 and 19.9 to 64.2 mg/kg, respectively. A linkage map available for the population was used for mapping quantitative trait loci (QTL) for grain Fe and Zn accumulation. The QTL analysis led to identification of 2 QTL for grain Fe on chromosomes 2A and 7A and 1 QTL for grain Zn on chromosome 7A. The grain Fe QTL were mapped in marker interval *Xwmc382-Xbarc124* and *Xgwm473-Xbarc29*, respectively, each explaining 12.6% and 11.7% of the total phenotypic variation and were designated as *QFe.pau-2A* and *QFe.pau-7A*. The QTL for grain Zn, which mapped in marker interval *Xcfd31-Xcfa2049*, was designated as *QZn.pau-7A* and explained 18.8% of the total phenotypic variation.

Key words: *biofortification, grain Fe, grain Zn, QTL mapping, T. boeoticum*

The nutritional health of humans is dependent primarily on plant foods either directly or indirectly (DellaPenna 1999). During the past 40–50 years, the major emphasis of plant breeding programs had been to increase crop productivity for meeting the calorific requirements of the world population. Despite a linear increase in food production over years, nearly half of the world population, though with adequate staple food intake, suffers with the deficiency of vitamin A, iron (Fe), and zinc (Zn). This nutritional deficiency has been termed as hidden hunger (Welch and Graham 1999). Among the most widespread nutrient deficiencies, the Fe deficiency is estimated to affect more than 2 billion people worldwide and the suboptimal Zn nutrition is more common than previously believed (Stoltzfus and Dreyfuss 1998). In India alone, the prevalence of anemia ranges from 47 to 98% in different states (FAO 1998). Biofortification of staple food crops for enhanced micronutrient content through genetic manipulations is the best option available to alleviate hidden hunger with little recurring costs (Welch and Graham 2004; Monasterio et al. 2007).

Availability of useful variability in the germplasm and understanding of its genetic architecture are the prerequisites for a breeding program aimed at biofortification of crop plants. Realizing the importance of biofortification, several studies were undertaken for the evaluation of germplasm and advance breeding lines for grain Fe and Zn content (Cakmak et al. 2000; Monasterio and Graham 2000; Chhuneja et al. 2006; Morgounov et al. 2007; Rawat et al. 2008), but the variability for grain Fe and Zn is limited in the modern day varieties of wheat. However, some wild species including *Triticum boeoticum*, *Triticum monococcum*, *Triticum dicoccoides*, *Aegilops tauschii*, and *Aegilops speltoides* have a wider range of grain micronutrient density (Cakmak et al. 2000; Monasterio and Graham 2000; Chhuneja et al. 2006;

Rawat et al. 2008). Synthetic amphiploids developed by crossing *Triticum durum* and *Ae. tauschii* (Mujeeb-Kazi 1995), also showed wide variability for grain Fe and Zn concentrations (Calderini and Monasterio 2003). So far, only a few studies have focused on studying the genetics of accumulation of micronutrients in the grains of major cereals like wheat and rice (Stangoulis et al. 2007; Shi et al. 2008). Understanding the genetic basis of accumulation of micronutrients in the grains and mapping of the quantitative trait loci (QTL) will provide the basis for devising the plant breeding strategies and for improving grain micronutrient content through marker-assisted selection.

We analyzed several accessions of *T. boeoticum* and *T. monococcum* for grain Fe and Zn content. One of the accessions of *T. boeoticum* (designated as pau5088) had relatively higher grain Fe and Zn content. The present communication describes the mapping of QTL for grain Fe and Zn in a recombinant inbred line (RIL) population derived from the cross *T. boeoeticum* (pau5088)/*T. monococcum* (pau14087). This RIL population has been used for generating A genome–specific linkage map (Singh et al. 2007) and for mapping disease resistance genes (Chhuneja et al. 2008).

Materials and Methods

Plant Material

The plant material used for mapping of the QTL for grain Fe and Zn consisted of a set of 93 RILs, derived from a cross *T. boeoticum* accession pau5088/*T. monococcum* accession pau14087 (hereafter referred to as *Tb5088* and *Tm14087*, respectively) through single-seed descent method. Detailed information on these accessions and molecular linkage map generated using this population was described by Singh et al. (2007) and is available at GrainGenes (http://wheat.pw.usda.gov/report?class=mapdata&name=T.%20boeoticum%20x%20monococcum). The parents and the RILs were planted in 1.5-m row, with row–row spacing of 50 cm at 2 locations, Punjab Agricultural University (PAU), Ludhiana (30°52′N, 75°56′E), India, and Indian Institute of Technology (IIT), Roorkee (29°52′N, 77°53′E), India, during 2004–05 and 2005–06. These environments hereon are referred to as PAU2005, PAU2006, IIT2005, and IIT2006, respectively. Standard agronomic practices were followed for raising the RIL population at both the locations. Seeds of individual lines were harvested manually and hand threshed to avoid any soil contamination. The phenotypic data for each RIL, at both the locations over both the years, were recorded as presented below and average of 4 values taken as pooled data. Hundred-seed weight of the parents and each RIL were recorded from the crop raised at PAU, Ludhiana, by weighing 100 grains on an electronic balance with a weighing precision of 0.1 mg.

Micronutrient Assaying

Because the parental lines and the RIL population had brittle rachis, the individual RILs and the parents were harvested as and when these matured. The seeds were removed manually ensuring no contact with any metallic containers and stored in glassine bags until analyzed. The grain Fe and Zn contents were analyzed within 6 months of harvesting during both the years. To ensure consistency in micronutrient analyses, the seeds from every lot were divided into 3 parts and analyzed as 3 replicates. The estimation of Fe and Zn for seeds from both the locations was done at IIT, Roorkee, following the protocol of Zarcinas et al. (1987). Briefly, the grains were washed quickly with 0.1 N HCl to remove any surface contaminants and dried in hot air oven at 80 °C. The washed grains (0.5 g) were digested in a mixture of 2 parts of concentrated nitric acid and 1 part perchloric acid (analytical reagent grade, Merck) until clear white residue was obtained. Required volume was made after the completion of digestion process, and digests were analyzed with atomic absorption spectrophotometer (GBC Avanta Garde), and the grain Fe and Zn concentrations were computed as milligrams/kilogram seed.

Statistical Analysis

Pearson correlation coefficient was calculated for all the 4 environments to test the correspondence of Fe and Zn data in different environments as well as to study correlation, if any, between Fe and Zn accumulation in the grains of the RIL population. Pearson correlation coefficient was also estimated for Fe and Zn and the 100-grain weight (100GW) recorded at PAU during 2005 and 2006. Student's *t*-test was used to test the significance of the correlation coefficient.

QTL Mapping

The grain Fe and Zn data for all the 4 environments individually and the pooled data were used for detecting the QTL governing grain Fe and Zn concentrations in this population. The positions and effects of QTL were determined following composite interval mapping (CIM) using the software QTL CARTOGRAPHER v. 2.5 (Wang et al. 2007). The significant threshold logarithm of the odds (LOD) scores for detection of the QTL were calculated based on 1000 permutations at $P \leq 0.05$ (Churchill and Doerge 1994). CARTOGRAPHER *Z* map QTL, Model 6, with a window size of 10 cM was used for the CIM. The number of markers for the background control was set to 5. Proportion of observed phenotypic variation explained due to a particular QTL was estimated by the coefficient of determination (R^2) using maximum likelihood for CIM.

Results

Grain Fe and Zn Content

Micronutrient analyses of the parental lines showed that *Tb5088* had relatively higher grain Fe and Zn concentration than *Tm14087* (Table 1). For *Tb5088*, the grain Fe and Zn concentrations, averaged over the 4 environments, were 40.1 and 44.6 mg/kg, whereas in *T. monococcum*, the average concentrations were 23.8 and 29.2 mg/kg, respectively. A wide range of variation was observed in the RIL

Table 1. Grain Fe and Zn concentration (milligrams/kilogram) of the parental accessions and the RIL population in 2 environments over 2005 and 2006

Environment	Parents		RILs (range)	Mean	Standard error$_{mean}$
	Tb5088	*Tm14087*			
Fe (mg/kg)					
IIT2005	39.6	22.5	17.5–70.9	30.3	1.2
IIT2006	37.8	24.5	16.3–69.2	30.0	1.2
PAU2005	38.9	23.6	16.6–69.0	33.3	1.2
PAU2006	44.1	24.6	13.1–69.4	32.9	1.3
Mean	40.1	23.8	17.8–69.7	31.6	1.2
Zn (mg/kg)					
IIT2005	43.8	29.7	17.1–64.3	31.9	1.2
IIT2006	48.4	31.4	18.8–63.5	31.9	1.2
PAU2005	41.9	28.6	18.0–69.0	33.1	1.2
PAU2006	44.6	27.2	16.8–60.0	32.2	1.2
Mean	44.6	29.2	19.0–64.2	32.4	1.2
100GW (g)					
PAU2005	0.64	1.08	0.30–2.16	1.1	—
PAU2006	1.16	1.32	0.45–2.41	1.5	—

population at both the locations for both the years. The population showed continuous distribution but was skewed toward lower levels of micronutrients (Figure 1), and transgressive segregants were observed for both Fe and Zn concentrations (Table 1, Figure 1).

Correlation Analysis

To study the effect of the environment on grain Fe and Zn concentration in the RIL population, Pearson's correlation coefficient (*r*) was determined for all the data sets. Correlation analyses showed that the grain Fe content of all the 4 locations was highly consistent with "*r*" ranging from 0.87 to 0.96 (Table 2). It was also true for grain Zn content with *r* ranging between 0.82 and 0.97. However, no significant correlation was observed between Fe and Zn concentrations in the grains, indicating that grain Fe and Zn accumulation may be controlled by different loci (Table 2). Micronutrients in the grains are concentrated in the aleurone layer, and the diploid primitive and wild wheats have a smaller grain size compared with the cultivated wheat. There is a concern that any higher micronutrient concentration could be due to smaller grain size (concentration effect) rather than a real high micronutrient density (content) in wild species. The correlation coefficients between 100GW and the grain Fe and Zn concentrations were found to be nonsignificant with *r* varying between 0.0 and 0.15 indicating that no relation between 100GW and Fe and Zn concentrations in the grains of the RIL population.

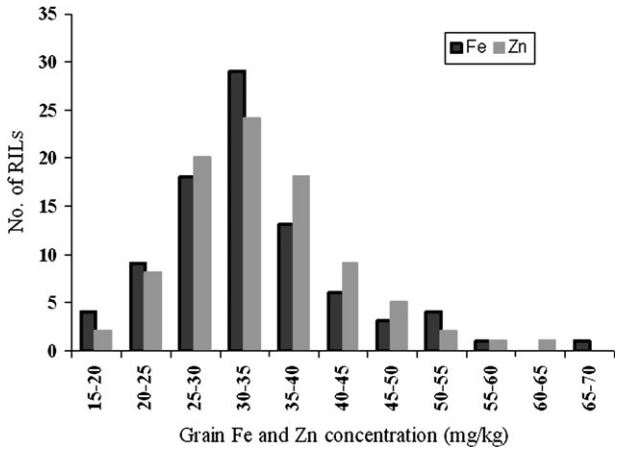

Figure 1. Distribution of *T. boeoticum*/*T. monococcum* RIL population for grain Fe and Zn concentration based on pooled data of 4 environments, PAU2005, PAU2006, IIT2005, and IIT2006. Average grain Fe concentrations (milligrams/kilogram) in *Tb5088* and *Tm14087* were 40.2 and 23.8, respectively, and the average grain Zn concentration (milligrams/kilogram) was 44.5 for *Tb5088* and 29.1 for *Tm14087*.

QTL Analysis for Grain Fe and Zn Concentration

A framework linkage map, based on 169 simple sequence repeat and restriction fragment length polymorphism loci (Singh et al. 2007), was used for mapping the grain Fe and Zn in a set of 93 RILs. The data for the individual environments (IIT2005, IIT2006, PAU2005, and PAU2006) and the average of all the 4 environments were used for detection and mapping the QTL controlling grain Fe and Zn concentrations. For grain Fe concentration, 2 significant and 1 suggestive QTL were detected (Table 3, Figure 2), whereas for grain Zn concentration, 1 significant and 1 suggestive QTL were detected (Table 3, Figure 3). The 2 significant QTL for the grain Fe map on chromosomes 2A and 7A in the marker intervals *Xwmc382-Xbarc124* and *Xgwm473-Xbarc29*, respectively (Table 3, Figure 4). The suggestive QTL was also detected on chromosome 7 in the marker interval *Xcfd31-Xcfa2049* (Table 3). The QTL on 2A, designated as *QFe.pau-2A*, had LOD scores of 3.7, 3.6, 2.7, 2.1, and 3.3, based on the grain Fe content measured in the environments IIT2005, IIT2006, PAU2005, PAU2006, and the pool data, respectively, with the respective coefficient of determination (R^2) values of 14.3, 13.8, 10.0, 8.0, and 12.6 (Table 3, Figure 2). Likewise, the QTL on chromosome 7A, designated as *QFe.pau-7A*, was detected at LOD scores of 2.3, 3.3, 2.4, 3.3, and 3.2 with respective R^2 values of 8.0, 12.6, 10.0, 11.7, and 11.7 for the environments IIT2005, IIT2006, PAU2005, PAU2006, and the pooled data, respectively (Table 3). The QTL *QFe.pau-2A* and the suggestive QTL in the marker region *Xcfd31-Xcfa2049* had positive alleles (allele that increases grain Fe concentration) from *T. boeoticum*, whereas the QTL *QFe.pau-7A* had a positive allele from *T. monococcum* (Table 3).

For grain Zn concentration, both the significant and the suggestive QTL mapped on chromosome 7A in the marker intervals *Xcfd31-Xcfa2049* and *Xgwm473-Xbarc29*, respectively (Table 3, Figure 3). The QTL mapped in the marker interval *Xcfd31-Xcfa2049* (Figure 4) was detected at LOD

Table 2. Pearson correlation coefficients between grain Fe and Zn concentrations and grain weight in the RIL population

	ZnIIT2005	ZnIIT2006	ZnPAU2005	ZnPAU2006	FeIIT2005	FeIIT2006	FePAU2005	FePAU2006	GW2005
ZnIIT2005	1								
ZnIIT2006	0.97**	1							
ZnPAU2005	0.93**	0.88**	1						
ZnPAU2006	0.93**	0.92**	0.82**	1					
FeIIT2005	0.13	0.15	0.09	0.15	1				
FeIIT2006	0.17	0.18	0.15	0.19	0.93**	1			
FePAU2005	0.08	0.11	0.06	0.11	0.96**	0.87**	1		
FePAU2006	0.13	0.14	0.10	0.15	0.91**	0.91**	0.90**	1	
GW2005	0.03	0.04	0.00	0.09	0.15	0.10	0.14	0.12	1
GW2006	0.14	0.10	0.13	0.10	0.04	0.02	0.04	0.05	0.63

**Significant at $P < 0.01$.

scores of 4.4, 3.2, and 4.2 with respective R^2 values 21.1, 14.4, and 18.8 for the environments IIT2005, IIT2006, and the pooled data, respectively (Table 3, Figure 3). This QTL has been designated as *QZn.pau-7A*. The suggestive QTL for grain Zn concentration maps in the same region where QTL for grain Fe concentration mapped, and the major grain Zn QTL was observed to be suggestive QTL for grain Fe concentration (Table 3, Figure 3). The QTL *QZn.pau-7A* has a positive allele from *T. boeoeticum*, whereas the suggestive QTL in the marker region *Xgwm473-Xbarc29* has a positive allele from *T. monococcum* (Table 3).

Discussion

So far, only 1 study has reported the genetics of accumulation of Zn in the wheat grains (Shi et al. 2008), but no report is available on the genetics of accumulation of Fe in wheat grains. The parental lines used by Shi et al. (2008) had grain Zn concentration of 34.4 (Hanxyuan 10) and 25.9 (Lumai 14) mg/kg, and the doubled-haploid (DH) population generated from this cross showed transgressive segregation, with some DHs having grain Zn as high as 50.8 mg/kg. The cultivated hexaploid wheat germplasm has a narrow range for grain Fe and Zn concentrations. Morgounov et al. (2007) reported grain Fe concentration in the range of 39–48 mg/kg in a set of 25 spring wheat cultivars and 34–43 mg/kg for 41 winter wheat cultivars with the exception of 1 spring wheat cultivar, Chelyaba, that had grain Fe concentration of 56 mg/kg. Similarly, the Zn concentrations reported in the winter wheat and the spring wheat sets were 23–33 and 20–39 mg/kg, respectively. Although the grain Fe and Zn concentrations in *Tb5088* and *Tm14078* were in the same range as reported for the cultivated hexaploid wheat germplasm (Morgounov et al. 2007), the grain Fe and Zn concentration in *Tb5088* was almost double than in *Tm14087*. A few RILs had grain Fe and Zn concentration of 65 and 60 mg/kg, respectively. The transgressive segregation in the RIL population is an indication of presence of different sets of genes in the parental lines for the target traits, which is corroborated by QTL analysis. Of the 2 significant QTL detected for grain Fe, one had positive allele in *T. boeoticum* on chromosome 2A and the other had positive allele from *T. monococcum* on chromosome 7A (Table 3). A suggestive QTL in the marker region *Xcfd31-Xcfa2049* has a positive allele from *T. boeoticum*. The QTL for grain Fe and Zn mapped in the present study, though explaining only 25–30% of the total phenotypic variation, are novel. These QTL, explaining relatively little of the total phenotypic variation, could be due to probable complexity of inheritance, the smaller population size, or the unaccounted QTL.

Table 3. Summary of the QTL for grain Fe and Zn concentrations in the RIL population detected using CIM

			IIT2005		IIT2006		PAU2005		PAU2006		Pooled		Favorable
Chromosome	Marker interval	Position (cM)	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	allele
QTL for grain Fe													
2A	*Xwmc382-Xbarc124*	23.6	3.7	14.3	3.6	13.8	2.7	10.0	2.1	8.0	3.3	12.6	Tb
7A	*Xgwm473-Xbarc29*	153.8	2.3	8.0	3.3	12.6	2.4	10.0	3.3	11.7	3.2	11.7	Tm
7A	*Xcfd31-Xcfa2049*	72.6	1.7	6.0	1.7	5.0	1.9	10.0	1.8	6.0	1.9	7.0	Tb
Threshold LOD values			2.6		2.7		2.7		2.7		2.7		
QTL for grain Zn													
7A	*Xcfd31-Xcfa2049*	72.6	4.4	21.1	3.2	14.4	2.2	8.1	1.6	6.0	4.2	18.8	Tb
7A	*Xgwm473-Xbarc29*	153.8	1.8	7.0	2.7	11.8	3.5	14.7	1.4	6.0	2.0	9.0	Tm
Threshold LOD values			2.7		2.7		2.7		2.7		2.6		

Tb and Tm refer to *T. boeoticum* and *T. monococcum* alleles.

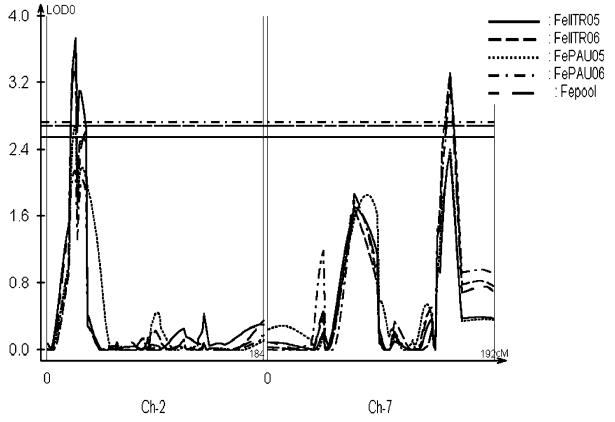

Figure 2. CIM for grain Fe concentration in the RIL population based on environments IIT2005, IIT2006, PAU2005, PAU2006, and pool data.

Shi et al. (2008) detected as many as 4 QTL for grain Zn concentration (milligrams/kilograms) and 7 (including these 4) for grain Zn content (micrograms/grain). The QTL detected by Shi et al. (2008) on chromosome 7A, explaining the highest level of phenotypic variation, maps in the same region where a suggestive QTL is mapping in the present study. In our group, several interspecific derivatives with high grain Fe and Zn have been generated from crosses with *Aegilops kotschyii*. These derivatives were observed to have Triticeae group 2 chromosome introgression for morphological and molecular markers (Tiwari VK, Rawat N, and Dhaliwal HS, unpublished data), suggesting the presence of an ortholog of *QFe-2A* in *Ae. kotschyi*. In rice, using a DH population, Stangoulis et al. (2007) mapped 3 QTL for grain Fe accumulation on chromosomes 2S, 8L, and 12L and 2 QTL for grain Zn on chromosomes 1L ad 12L. The region where grain Zn QTL was mapped on rice chromosome 1 is orthologous to wheat chromosome 7. The total phenotypic variation for grain Fe and Zn concentrations explained by

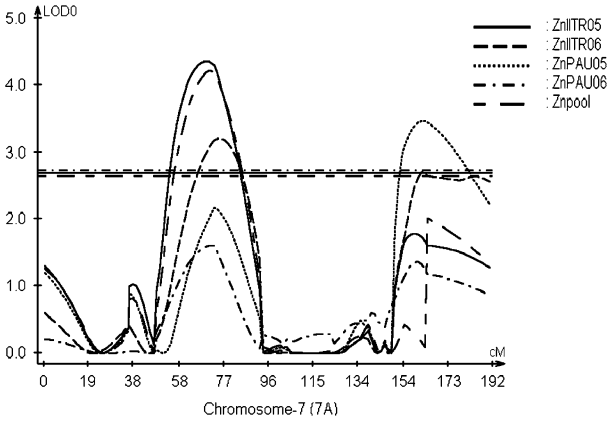

Figure 3. CIM for grain Zn concentration in the RIL population based on environments IIT2005, IIT2006, PAU2005, PAU2006, and pool data.

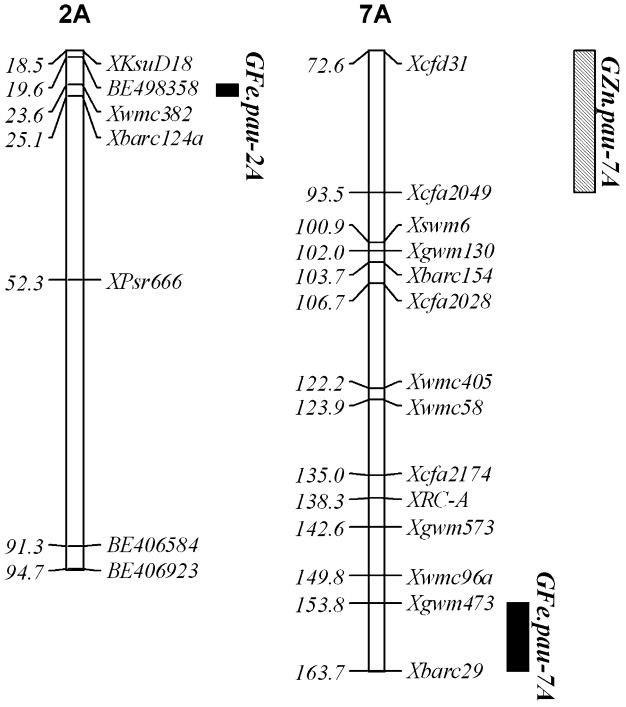

Figure 4. Partial linkage maps of chromosomes 2A and 7A showing chromosomal location of significant QTL for grain Fe and Zn concentrations. Values on the left of the map are the centimorgan distances from terminal end of the short arm of the linkage map. For complete linkage map, readers may refer to Singh et al. (2007) or (http://wheat.pw.usda.gov/report?class=mapdata&name=T.%20boeoticum%20x%20monococcum).

the mapped QTL is relatively low. There is a possibility that the QTL with major effect are not yet detected from this population.

In several studies on the micronutrient analyses in different crops and populations, high positive correlation has been reported between Fe and Zn content in grains and other tissues. Accumulation of grain Fe and Zn, however, did not show any correlation in the present study. This can be explained from the fact that QTL for grain Fe and Zn in *T. boeoeticum* map on different chromosomes (Table 3). The notion, that accumulation of Fe and Zn in grains is positively correlated (Shi et al. 2008), may not be true as all the studies were based on estimation of grain Fe and Zn in a set of fixed lines (Morgounov et al. 2007) rather than in the segregating populations. One study in rice (Stangoulis et al. 2007), however, using a DH population reported a highly significant positive correlation between grain Fe and Zn concentration. This may be true for certain loci, as is evident from the present study as well. In *T. monococcum* a significant QTL for grain Fe and a suggestive QTL for grain Zn map in the same chromosomal region *Xgwm473-Xbarc29* (Table 3). Another notion that the grain weight may affect the grain Fe and Zn concentrations may also not be true, as in the present study no correlation was observed between grain size in RILs and the Fe and Zn accumulation. The

higher micronutrient concentration in the wild species has often been thought as a result of dilution/concentration effect due to smaller seeds, but identification of some of the RILs with bolder seeds and higher micronutrient content (RIL11, 20, 38, 46, and 57 in the present study) refutes this concept as well. This is further corroborated by the work of Shi et al. (2008) who showed a complete overlap of QTL for grain Fe and Zn concentrations (milligrams/kilograms) and the contents (milligrams/grain). This, in fact, is encouraging for attempting the transfer of grain Fe and Zn QTL from wild species to the cultivated wheat. The high grain Fe and Zn QTL from *T. boeoticum* can be transferred to bread wheat using *T. durum* as a bridging species. In this approach, chromosomally stable tetraploid plants become available in the first backcross generation (Chhuneja et al. 2008). The BC_1F_1 plants can be analyzed with the markers flanking grain Fe and Zn QTL, and the positive plants can be analyzed for grain Fe and Zn. The tetraploid plants with introgression for target regions from *T. boeoeticum* and having high grain Fe and Zn concentration can be used for transferring the traits to bread wheat. This approach will be most suitable for validation of QTL and expression of the trait in higher ploidy levels.

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